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Karyotypes of nine Venezuelan annual killifishes (Cyprinodontidae), with comments on karyotype differentiation in annual killifishes

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Karyotypes of nine species of Venezuelan annual killifishes were compared. Karyotypic differences were found between species and between some genera. All *Rachovia* species have a diploid number of 44. *Rachovia maculipinnis* and *R. brevis* differ markedly from one another in metacentric chromosome number (20 and 12 respectively). There are 10 metacentric chromosomes in the complement of both *R. hummelincki* and *R. pyropunctata*.

No karyotypic differences were found between *Austrofundulus transilis* and *A. limnaeus* (2N = 44, metacentrics = 12). Several populations of the latter, although divergent in male color patterns, did not differ karyotypically. All species examined, except *Rivulus stellifer*, departed from the presumptive ancestral teleost diploid number of 48. *Rivulus stellifer* differs from this ancestral karyotype by a number of pericentric inversions resulting in a NF > 48.

The degree of chromosomal variation reported here and in the literature appears not to be as high among the New World as among the Old World annuals. The difference in karyotypic variability of these two groups, having similar life histories and reproductive strategies, suggests that habitat stochasticity, associated with annual reproductive strategies, may not have been the primary force mediating chromosomal differentiation in both of these groups of annual fishes.

Introduction

Annual killifishes (family Cyprinodontidae) in both the Old and New World live in temporary seasonal habitats and each generation ordinarily must complete its life cycle within a few months. During the dry season populations consist only of estivating eggs. Each successive generation must hatch, mature and spawn over the course of a few months in concert with unpredictable, seasonal wet and dry cycles of the habitat (Myers, 1952). Both Old and New World annual killifishes have radiated into highly variable seasonal habitats and several workers have hypothesized that this high degree of environmental stochasticity has lead to high levels of

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differentiation and polymorphism among Old World annual killifishes (Myers, 1952; Scheel, 1966a, 1972b; Wourms, 1972). Chromosome numbers and morphologies are known to be highly variable between and within species of Old World annuals (Scheel, 1966a, 1972b, 1990). New World annuals have been much less well characterized karyotypically. If environmental variability is a general factor, among annual killifishes, resulting in high levels of karyotypic polymorphism, then, these effects might be assumed to be equally operative among both New and Old World annuals. If this hypothesis is true, then, karyotypic variation among the New World annual killifishes should parallel, in extent, that known to exist among those from the Old World.

Annual killifish are restricted to temperate and tropical Africa and South America and can be divided into Old and New World groups with no currently recognized genera in common (Scheel, 1968). Scheel (1968) classified both Old World and New World forms together in the subfamily Rivulinae. A more recent classification by Parenti (1981) placed the New World killifishes into the family Rivulidae and the Old World forms into a sister family, Aplocheilidae. There are approximately 250 annual and non-annual species of which roughly 100 are from the New World (Scheel, 1966b). Of interest here is a group of related annual rivulins found in the Orinoco and Lake Maracaibo Basins of Venezuela. These have been revised taxonomically by Taphorn & Thomerson (1978).

The karyotypes of nine previously unstudied Venezuelan annual species were examined in order to address several related questions. What is the nature of karyotypic variation in this group? How does this variation (along with that reported by others) for New World annuals compare to that known to exist among Old World species? Can species within closely related complexes be distinguished on the basis of karyotype and, can genera be separated on this basis? Do observed inter- and intrapopulational differences in male coloration parallel chromosomal variation?

Material and methods

Specimens for this study were collected by JET and DCT in the Orinoco and Maracaibo Basins, Venezuela during 1986 and 1987. Fish were shipped to Virginia Polythehnic Institute & State University and held in aquaria until processing.

Table 1. Karyotypes of 19 species of New World Annual Killifish. **2N,** diploid count; **A,** diploid arm number; **M,** metacentric chromosome count; **S,** source. Source numbers: 1, this study; 2, Post, 1965; 3, Scheel, 1972; 4, Olivera et al., 1988.

Species	2N	NF	A	SM	M	S	Number of specimens	
Pterolebias zonatus	42	60	30	6	12	1	6♂	7♀
P. hoignei	46	56	36	4	6	1	6♂	10♀
P. longipinnis	40	40	—	—	—	2,3	—	—
P. peruensis	54	90	—	—	—	3	—	—
Rachovia brevis	44	70	18	14	12	1	2♂	1♀
R. pyropunctata	44	62	26	8	10	1	2♂	4♀
R. hummelincki	44	64	24	10	10	1	3♂	6♀
R. maculipinna	44	76	12	12	20	1	3♂	4♀
Austrofundulus limnaeus	44	72	16	16	12	1	8♂	6♀
A. transilis	44	72	16	16	12	1	5♂	4♀
Rivulus stellifer	48	74	22	8	18	1	9♂	1♀
Cynolebias bellotti	48	—	—	—	—	2,3	—	—
C.nigripinnis	48	—	—	—	—	2,3	—	—
C. whitei	48	92	—	—	—	2	—	—
C. whitei	46	92	—	—	—	3	—	—
C. cheradophilus	40	—	—	—	—	4	—	—
Leptolebias ladiges	48	—	—	—	—	2	—	—
Cynopoecilus melanotaenia	48	—	—	—	—	2	—	—
C. melanotaenia	44	52	—	—	—	3	—	—

Sample sizes and collection locations are given in Table 1 and Appendix I.

Mitotic karyotypes were prepared from gill epithelium and meiotic spreads were prepared from testis using the technique of Kligerman & Bloom (1977) as modified by Turner et al. (1985) for all species except *R. stellifer*, whose karyotypes were prepared from scale epithelium and caudal fin tissue of living specimens using a modification of the method of Ramirez (1980). Slides were examined and photographed at a magnification of 1,000 X. Negatives were enlarged approximately 10 X during printing. Two chromosome spreads per specimen were photographed.

When counts of chromosome numbers did not agree, several more spreads were photographed and analysed until at least three were found whose counts did agree. Only spreads of similar degrees of contraction were photographed.

Karyotypes were prepared from prints using a modification of the system of Levan (1964). Individual chromosomes were classed according to arm-length ratios. For this study, only gross distinctions were made due to the small size of chromosomes and the variable resolution of submetacentrics. Chromosomes with arm ratios of less than 1.7 were considered to be metacentric and those with ratios of 3.0 or more were considered acrocentric. Chromosomes with arm length ratios between 1.7 and 3.0 were classed as submetacentric. This classification scheme reduces subjective judgement in matters of resolution and results in a conservative estimate of chromosomal variation.

Results

Chromosome numbers and morphologies are summarized in Table 1. Representative karyotypes for each species other than *Pterolebias hoignei* and *P. zonatus* are presented in Figure 1. Karyotypes for the *Pterolebias* species are reported elsewhere (Elder et al., 1991). Submetacentric chromosome numbers reported are the maximum number detected for a particular species. Apparent intraspecific variation in submetacentric numbers may be artifactual. Our estimates of karyotypic divergences are based on diploid numbers and metacentric chromosome counts alone.

Within the genus *Rachovia* all species have a diploid number of 44. Differences were found in the number of metacentric chromosomes of *R. maculipinna* and *R. brevis* (Fig. 1a-b) (20 and 12 respectively). These karyotypes differed from those of *R. hummelincki* and *R. pyropunctata* in metacentric chromosome number. Both of the latter species have a diploid number of 44 and a metacentric chromosome count of 10 (Fig. 1c-d). Since no difference in chromosome number was found, karyotypic differences among the *Rachovia* species are probably the result of non-Robertsonian rearrangements. *Austrofundulus limnaeus* and *A. transilis* could not be distinguished on the basis of chromosome counts. No karyologic differences were detected among *A. limnaeus* color morphs or between allopatric *A. limnaeus* populations. *Rivulus stellifer* differed from the other New World species, karyotyped in this study, in both total chromosome number and in metacentric chromosome number as well.

Discussion

On a specific level, chromosomal divergence, while not as extensive as that found within the Old World group, does exist within the New World annuals.

The genus *Rachovia* has been recently revised (Taphorn & Thomerson, 1978) and is considered to be monophyletic. *Rachovia brevis* and *R. maculipinna* were regarded as sibling species which had diverged in allopatry as a result of the formation of the Andean mountain range. Though similar morphologically, *R. hummelincki* and *R. pyropunctata* were separated on the basis of consistent differences in male coloration and range disjunction. These two taxa are otherwise difficult to distinguish. *Rachovia hummelincki* is confined to xeric coastal areas in Colombia and Venezuela. *Rachovia pyropunctata* is found more inland within the moist conditions of the Lake Maracaibo Basin. The divergence of these two species is believed to have taken place within the last few thousand years (Taphorn & Thomerson, 1978).

The chromosomal data from the *Rachovia* sample agree well with the proposed sequence of divergence. The karyotypes of *R. maculipinna* and *R. brevis* have diverged considerably from each other and from those of the other two *Rachovia* species. The karyotypes of *R. hum-*

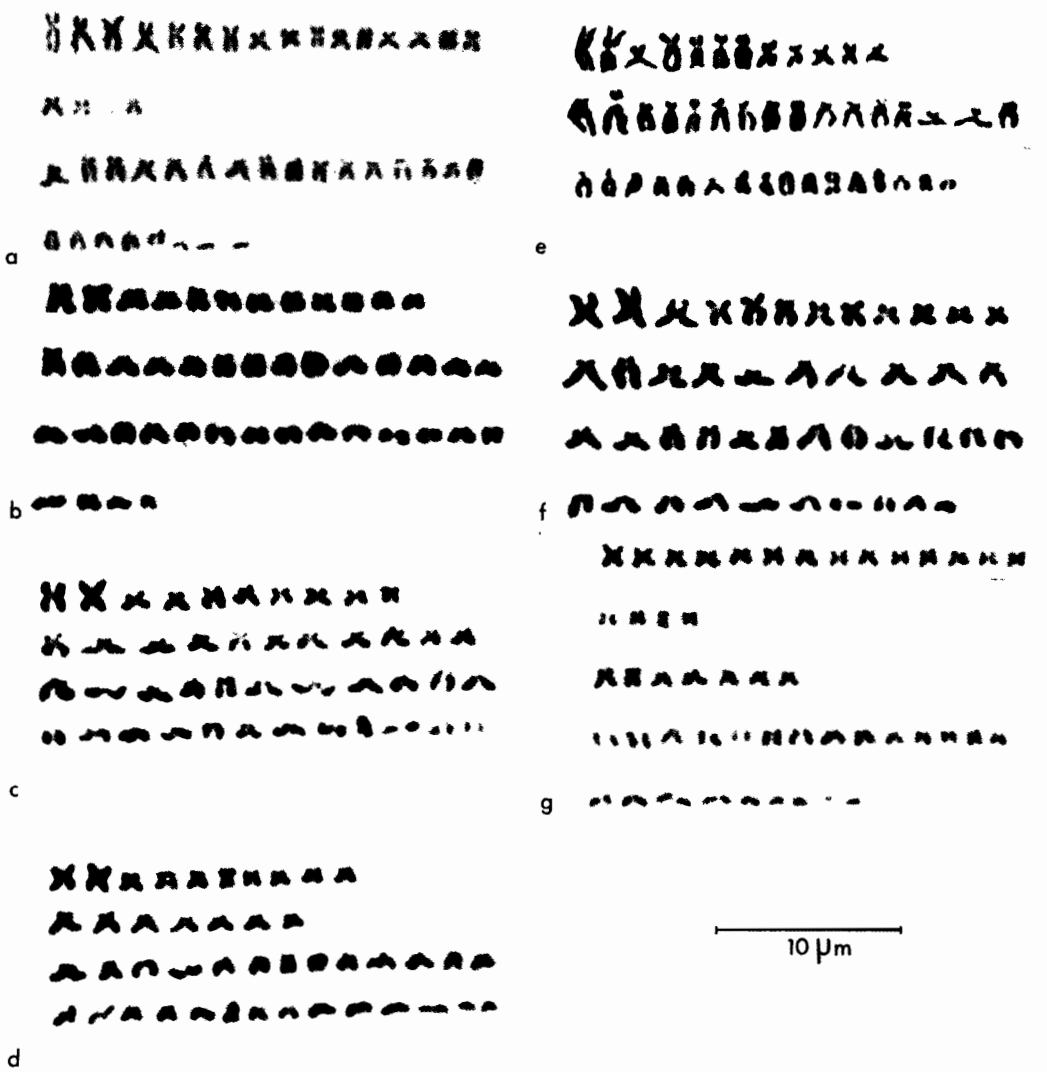

Fig. 1. Representative karyotypes of some Venezuelan annual killifishes. **a,** *Rachovia maculipinna*, **b,** *R. brevis*, **c,** *R. hummelincki*, **d,** *R. pyropunctata*, **e,** *Austrofundulus limnaeus*, **f,** *A. transilis*, **g,** *Rivulus stellifer*.

melincki and *R. pyropunctata* reveal no discernible differences. Since both the morphological and the chromosomal data fail to resolve these two presumptive species, the possibility that they are geographical races of a single species cannot be negated by our karyotype data. Their status awaits further clarification by other criteria.

Only two species of *Austrofundulus*, *A. transilis* and *A. limnaeus*, are currently recognized. These two species are morphologically distinct (Taphorn & Thomerson, 1978). *Austrofundulua transilis* seems to be fairly widespread in the central Orinoco llanos. *Austrofundulus limnaeus* has a much broader distribution. It ranges from the Colombian coastal llanos to the Amazon Basin of Guyana, but is not known from the Orinoco Basin (Taphorn & Thomerson, 1978). *Austrofundulus limnaeus* and *A. transilis* are not chromosomally distinguishible (Fig. 1e-f). Both have a diploid number of 44 and a metacentric chromosome count of 12. Taphorn & Thomer-

son (1978) pointed out that populations of *A. limnaeus* exhibit a high degree of inter- and intrapopulational variation in male coloration. Karyotypes of male color variants from three localities revealed no intra-variant or intrapopulational differences.

The genus *Austrofundulus* is closely related to *Rachovia*. Both genera share characteristics with *Rivulus stellifer*, a more primitive form, and are considered to be descended from a common rivulus-like ancestral type (Taphorn & Thomerson, 1978). Taphorn & Thomerson (1978) considered these genera discrete on the basis of their morphology. At the present level of resolution, all *Austrofundulus* share a common karyotype with *Rach. brevis*. The karyotypes of *Rach. hummelincki* and *Rach. pyropunctata* differ from the *Austrofundulus - Riv. brevis* karyotype by a single presumptive pericentric inversion. *Rachovia maculipinna* is the exception here, with a metacentric chromosome count of 20, requiring the fixation of many more non-Robertsonian rearrangements.

The presumed ancestral karyotype of teleost fishes is considered to be 48 small telocentric chromosomes (Kornfield, 1984). If this is the case, then the karyotype of *Riv. stellifer*, (Fig. 1g) with a diploid number of 48, is the most primitive in this group (note however, that there are 18 metacentrics in its karyotype, not a primitive trait). The karyotype for *Riv. stellifer* reported here is consistent with those reported for other *Rivulus* species (Scheel, 1972b). It should be noted that there is a large amount of karyotypic variation in this genus (diploid count ranges from 40 to 48 while arm numbers range from 54 to 92) (Scheel, 1972b). Considerable deviation from the presumed primitive karyotype, involving both Robertsonian and non-Robertsonian changes, is evident in *Rivulus, Rachovia* and *Austrofundulus*.

The role played by chromosomal mutation in the evolution of new species is unclear. Many closely related species differ karyotypically. This correlation has led to the development of models which assume that chromosomal changes are capable of causing barriers to gene flow between populations. Under these models, chromosomal changes may induce reproductive isolation and are therefore considered as causal agents of speciation (Sites & Moritz, 1987).

Because of lowered heterozygote fitness, a newly arising chromosome variant is unlikely to become fixed in a large population. Stochastic forces (genetic drift, etc.) may fix the new variant in the population if it arises in a deme of sufficiently small size (Sites & Moritz, 1987). Random drift, viability advantage of heterozygotes and meiotic drive (Wright et al., 1983) have all been implicated in the fixation of rare chromosome mutants within populations (Bengtsson & Bodmer, 1976). Kin founding has been shown to increase the chances that a chromosome rearrangement will be fixed, because of the higher initial frequency of the variant within the founding group than that which would exist in a population of unrelated individuals (Hendrick & Levin, 1984). Sexual selection may also increase the likelihood of the fixation of a rare mutant (Sites & Moritz, 1987). The canalization model of Bickham & Baker (1979) is based upon the assumption that chromosome rearrangements can form new gene linkage groups with adaptive advantages, which are preserved by selection during the divergence into new niches. Yet another line of thought suggests that reproductive isolation is caused by the disruption of coadapted gene complexes during meiosis as a result of chromosomal rearrangement. It has been suggested that separate demes may, through rearrangement, evolve different gene complexes which are not passed on to F1 or F2 hybrids (Shaw & Coates, 1983).

Other forms of chromosome mutation may contribute to karyotypic variation, yet have no direct effect upon reproductive isolation. For instance, in rodents, high degrees of variation have been observed at specific and generic levels in the number of heterochromatic arms of the karyotypes, and in the size and number of C-bands due to interstitial heterochromatin. The evolutionary role of such heterochromatic variation is speculative (Patton & Sherwood, 1983).

Chromosomal divergence within the New World annuals appears to be relatively conservative when compared to that existing within the Old World annuals. Karyotypes for the New World species studied here have diploid numbers ranging from 42 to 48 and metacentric chromosome counts ranging from 6 to 20. The observed numbers indicate karyotypic changes due to both Robertsonian and non-Robertsonian rearrangements.

Four Old World genera of the subfamily Rivulinae (*Nothobranchius, Fundulosoma, Aphyo-*

semion and *Epiplatys*) are known to consist either partially or wholey of annual species (Scheel, 1966a, 1990). Chromosome numbers and morphologies are highly variable among Old World species. Hybridization experiments between chromosome variants within the genus *Aphyosemion*, which contains both annual and non-annual species, produced sterile F1 males (Scheel, 1966a). Further study has disclosed a high degree of karyotypic variation within species in the Old World group. There are populations of annual *A. rubrolabiale* which have polymorphic karyotypes differing both by diploid number and by number of chromosome arms. Similar karyotype polymorphism is seen in the Old World Annual *N. kirki* (Scheel, 1990).

Such variation was also described for two aquarium strains of non-annual *A. labarrei* presumably derived from different natural populations (Scheel, 1972a). *Aphyosemion christyi*, another non-annual, exhibited diploid counts ranging from 18 to 30. Scheel (1971) postulated that this degree of variation could not be accounted for by a limited number of chromosomal mutations but must represent karyotypes finely adapted to specialized habitats. Robertsonian polymorphisms have been described in geographically separated annual killifish populations of *N. guentheri, N. foerschi, N. melanospilus* and *N. rachovii* (Ewulonu et al., 1985).

Overall, the Old World rivulins exhibit a high degree of both inter- and intraspecific karyotypic divergence. This is presumably due to the greater chance that chromosomal mutations will be stochastically fixed in small demes during species expansion within a highly variable, seasonal habitat than would be likely in groups having large effective population numbers.

If features of annual life history, especially the potential for frequent reduction in effective population size, are general forces, strongly influencing the extent of chromosomal variation among annual killifish, then the extent of variation within the New World annuals should roughly parallel that known to exist within the Old World annuals.

In order to test this hypothesis comparisons were made between the distributions of haploid numbers and the distributions of arm numbers for both groups. The distributions of karyotype numbers for the Old World annuals were taken from those reported by Scheel (1990). The distributions for the New World Annuals were derived from those reported here and in Scheel (1972b), Post (1965) and Olivera et al. (1988). Because of its robustness when dealing with unequal sample sizes, a Moses non-parametric test for equal distribution variances was used to detect differences between the Old and New World distributions.

The Moses test rejected the null hypothesis that the variance of chromosome numbers was the same in both groups (selected alpha = .05, W = 66 and P = .032). If stochastic forces were primarily and equally responsible for the divergences from a common ancestral karyotype for both Old and New World annuals, one would predict that their chromosome number distribution variances would be statistically indistinguishable. On this basis we conclude that different forces were active in the karyotypic evolution of each group and that chance alone could not have been the major force active in both groups.

Morphologically distinguishable populations of *Austrofundulus limnaeus* from three isolated locations reveal no karyotypic differences. Differences between genera in diploid number alone (42 to 48) within the New World annuals are not as great as within some genera from the Old World annuals (24 to 44 for *Nothobranchius* and 30 to 46 for *Aphyosemion*) (Scheel, 1990). This suggests that effects operating over a much longer time scale, such as allopatric speciation resulting from geographic isolation, are likely to have driven chromosomal differentiation within this group.

Central to general stochastic models of chromosome change in annual fishes is the presumption that low effective population sizes are a frequent occurrence in their evolutionary history. Supposedly, effective population sizes are small when wet seasons fail or are abbreviated. However, other annual fish adapations may significantly increase effective population sizes. The first of these is the "multiplier effect". The populations survive the dry season by means of estivating eggs which are arrested in development at one of three diapause stages (Wourms, 1972). Eggs of the New World annual *Cynolebias nigripinnis* have been documented to survive for three years under anoxic conditions (Scheel, 1968).

These adaptations ensure that not all eggs will hatch simultaneously and that the propor-

tion of eggs that are ready to hatch increases with time. This variation in hatching time prevents the loss of entire spawns to short lived wet periods which do not allow maturation and reproduction. This strategy increases the probability that some portion of the next generation will survive to reproduce (Wourms, 1972). This strategy also introduces the possibility of temporal gene flow across generations, thereby increasing effective population sizes and reducing the effectiveness of stochastic forces in generating chromosomal polymorphism. In addition, the physical isolation of local populations may be less severe than apparent at first glance. Wet season floods can cover vast areas and adult fishes from different pools may become thoroughly mixed before breeding.

Our data imply that the relative importance of stochastic and deterministic forces has been different in the chromosomal evolution of Old and New World annual killifishes. At the present, however, the biology of these fishes offers no readily apparent explanation for this observation.

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Appendix I: Field locations

M-87-1. *A. limnaeus, R. brevis, R. maculipinna, R. hummelincki.* Zulia, 2 km west of the "Y" at Guarero, toward Maricaibo, Colombia. Small prestamo south of the road.
M-87-2. *A. limnaeus, R. pyropunctata.* Zulia, 12 km southwest on Punto Arroyo Road from the intersection with Highway 6 (due north of Carrasquero).
M-87-3. *A. limnaeus, R. stellifer.* Zulia, prestamo on west fringe of Quisero.
M-87-4. *A. limnaeus.* Zulia, Small cano 22.6 km southeast of San Jose on the road going to the lake.
M-87-5. *A. limnaeus.* Falcon, small prestamo in the forest, 1.2 km north of Sanare, on the road to San Juan de los Cayos.
M-87-6. *A. limnaeus, R. pyropunctata, R. maculipinna.* Zulia, pozo in the southeast corner of the intersection of Hwy 1 and road to Ciudad Ojedes.
M-87-7. *A. limnaeus, R. pyropunctata.* Zulia, stock pond on the lake side of Hwy 3, 5.3 km north of Rio Machango and south of Bachaquero.
M-87-8. *A. limnaeus.* Zulia, stock pond on the lake side of Hwy 3, 6.6 km south of Rio Machango and north of Rio Misoa.
Km locations refer to marked kilometers along the Guanare - Guanarito Road, Edo. Portuguesa, Venezuela.
P. hoignei and *P. zonatus* collection locations are reported in Elder et al, 1991.

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